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Pharmacy and Allied Health Professions
Section of Medicinal Chemistry

June 14, 1984

Jet Propulsion Laboratory
California Institute of Technology
4800 Oak Grove Drive
Pasadena, CA 91103

ATTN: Dr. Dorothy Quinlan
Sr. Contract Negotiator

SUBJECT: Final Report on Subcontract 956837, "Development of a New Class of Chemical and Biological Ultrasensors: Ribonuclease Contamination and Control"

A. Ribonuclease Contamination

In order to define ribonuclease contamination, an assay for ribonuclease having picogram level sensitivity was established as defined by the scheme shown in Figure 1. In this assay, polycytidylic acid is digested by ribonuclease leading to smaller fragments of poly C that remain soluble after treatment of the sample with perchloric acid and lanthanum acetate. An absorbance measurement at 260 nm of the supernatant from the centrifuged sample measures the ribonuclease. A standard curve is shown in Figure 2.

Using this assay procedure, ribonuclease contamination was found to be significant in routine laboratory proteins, in particular, bovine serum albumin, lysozyme, catalase, and cytochrome C. This was confirmed by demonstrating a considerable reduction in this activity in the presence of phosphate buffer since phosphate inhibits ribonuclease. Ribonuclease contamination was not significantly encountered in routine laboratory glassware, plasticware, column surfaces, chromatographic particles, and buffer reagents, including airborne contamination. Some contamination could be introduced by fingerprints, however.

B. Chemical and Physical Techniques for Overcoming Ribonuclease Contamination

Ribonuclease contamination in routine laboratory proteins was overcome, at least below the sensitivity level of our assay, by treating the sample with iodoacetate or iodoacetamide, especially at an elevated temperature of 60-80 °C, either in a routine buffer or in the presence of 6 M urea or 6 M guanidine hydrochloride. This technique was quite powerful, eliminating ribonuclease activity even

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starting with a sample of ribonuclease itself. Ribonuclease contamination in fingerprints was overcome either by good laboratory technique, avoiding fingerprint contamination of equipment surfaces, or by heating the contaminated surfaces to 200 °C, or by washing the contaminated surface with 0.2% diethylpyrocarbonate in water.

Yours truly,

Roger W. Giese

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Professor
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RWG:cma

cc: R. McNeil, Jr.
I. Skurnick

RIBONUCLEASE ASSAY PROCEDURE

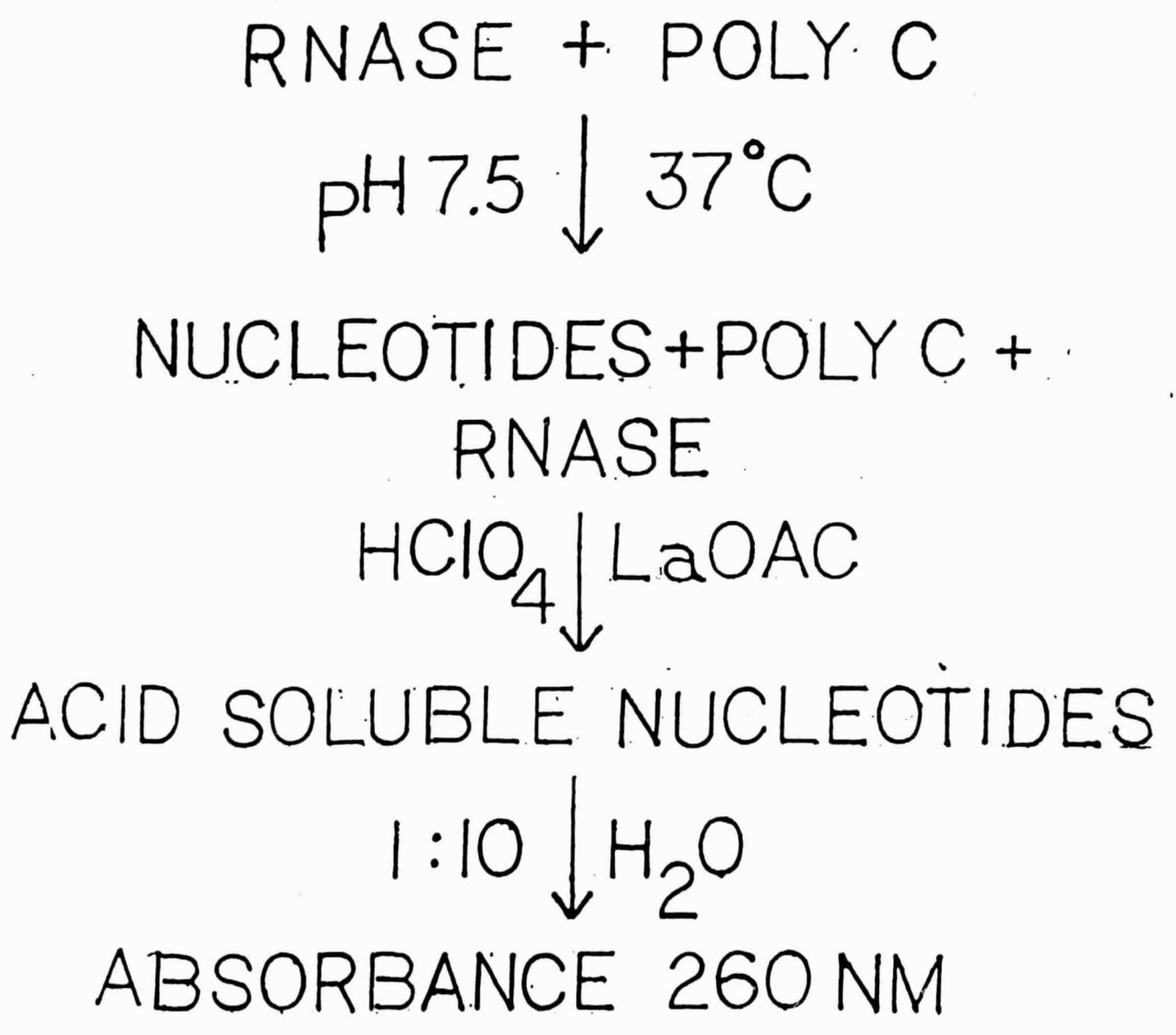
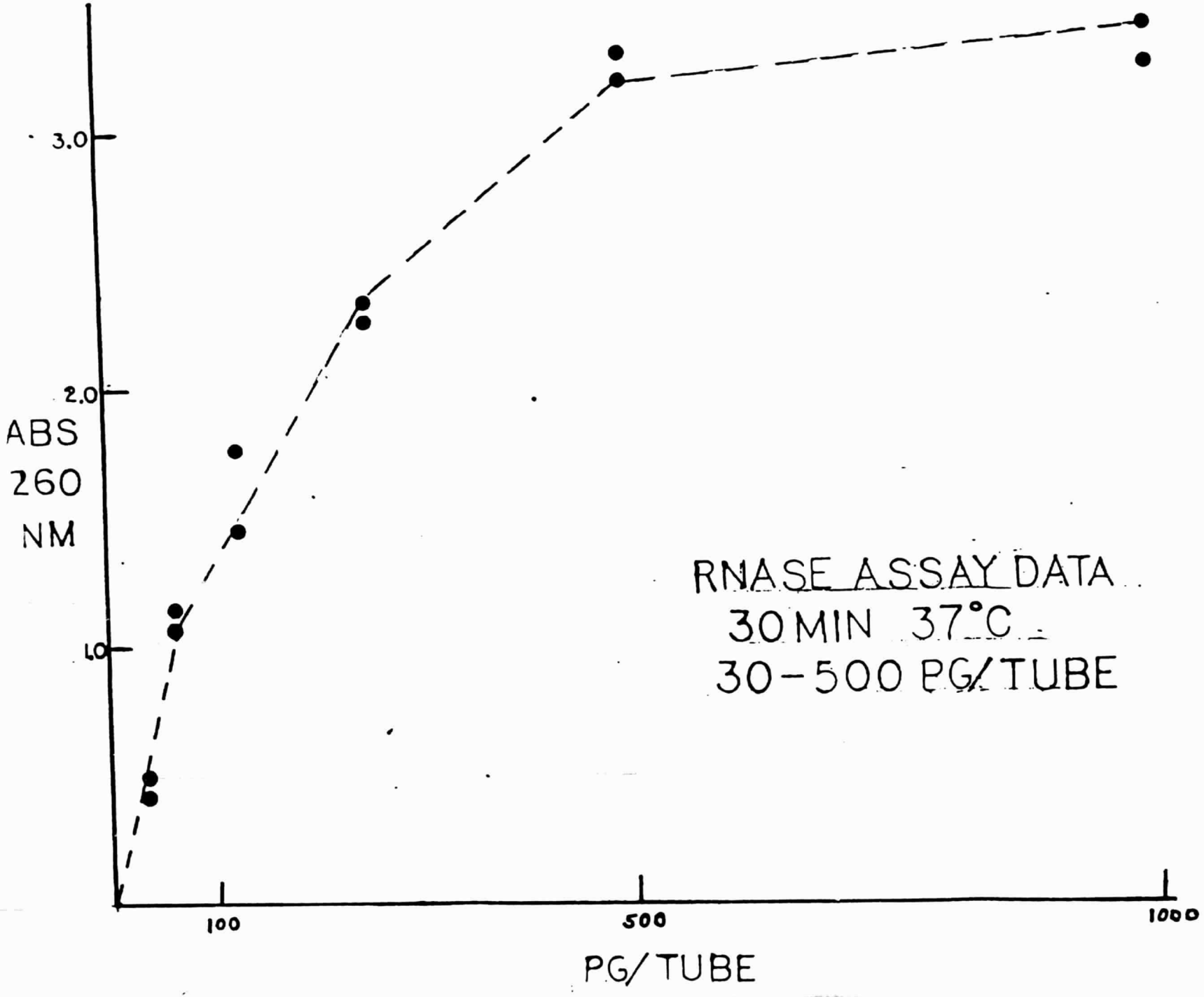


FIGURE 1

FIGURE 2


3.0
2.0
1.0
ABS
260
NM
100
500
1000
PG/TUBE
RNASE ASSAY DATA
30 MIN 37°C
30-500 PG/TUBE